SOME STUDIES IN ASEPSIS.[1]

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OCCASIONAL visits to operating rooms have impressed me with the fact that, to some extent, surgeons are inclined to overestimate the importance of small possible dangers, and to take more or less for granted absolute immunity from some others of greater magnitude. It was the unquestioning faith which some have shown in the instantaneous germicidal power of corrosive sublimate and other chemicals that gave me my first active interest in the general subject of asepsis and infection; and investigation of the actual value of a large number of these substances led me to consider other matters connected with operative work, including the danger of aerial infection, the sterilization of dressings and sponges, and the disinfection of the skin of the field of operation and of the surgeon's hands.

I shall never forget the look of utter consternation on the faces of all concerned when, one day, during an operation for hernia, I placed a sterile Petri dish upon a spot on the instrument table, about eight inches away from anything lying thereon, in the belief that the surgeon, by whose invitation I was to make certain observations, had arranged for the same with his colleagues. One would have supposed that that innocent dish was a seething mass of infection, fully prepared to disseminate the germs of septicæmia in all directions, even as a pinwheel throws its sparks. A hurried consultation was held, while I made my excuses and attempted to explain the absence of real danger. The result was the covering of that end of the table and the dish with a sterile towel. At this stage of the case, my culpable partner entered and explained matters, whereupon the towel was removed, the culture medium in the dish was exposed, and the operation proceeded. At the close of the operation, the dish was removed and incubated. The result demonstrated that upon each square inch of the dish and, inferentially, of the table and of the instruments thereon, and presumably of the field of operation, no less than 120 organisms, chiefly pus cocci, were deposited from the air in the course of an hour. It is not for me to say how much danger may reside in such a shower of bacteria. Frankly, I do not know, nor does there seem to be any unanimity of opinion on the part of those who have investigated the question of aerial infection; but it seems to me that the subject is not generally considered to be of such importance as the possibility of infection from sweat or from the introduction of an occasional bacterium from the superficial or deeper layers of the skin or from other sources. It has seemed to me that the danger of infection through the escape of droplets of sweat of a carefully prepared hand through an accidental puncture of a rubber glove is accorded undue weight. I have read numerous reports of experiments concerning the infectivity of sweat, and most of them have impressed me as unwarranted in their conclusions, owing to faulty premises and technique. In a recent article on the subject, it is dogmatically asserted, "The purest of sweat is impure; it is never sterile." With that statement I take issue. Six different times in my laboratory, sweat has been made to flow from well-cleaned, and so far as is possible, sterilized forearms and hands, encased in sterile glass cylinders heated by appropriate means; and in not a single instance could a bacterial growth be obtained. Moreover, injections thereof in fairly large amounts into animals—subcutaneously, intravenously, and intraperitoneally—were quite devoid of results. That there are bacteria in the various layers of the skin and in the hair-follicles there can be no doubt; but that they exist in the sweat-glands, from which the outflow of secretion would tend to bar them, is by no means clear. Indeed, I am informed by a number of our leading pathologists that an infection starting in a sweat-gland is exceedingly rare.

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Of far greater importance, it seems to me, is the danger of infection through saliva. Repeatedly have I seen surgeons, even in abdominal cases, talking directly into the wound. It has been demonstrated by Flügge, of Breslau, and by several others, that in ordinary conversation there is a constant throwing out of minute droplets of saliva, some of which are projected laterally several feet. They are expelled in great numbers in the use of words or syllables beginning with the consonants *d, k, p,* and *t,* the formation of which involves the sudden explosive liberation of air held in the mouth under pressure. They may be sent forth as numerously during whispering as with loud speech. Now, the mouth cavity is a singularly unclean place, for the secretions of the mouth are likely to be richer in bacteria than the foulest sewage, and these bacteria are largely staphylococci, diplococci, and streptococci, and are likely to be exceedingly virulent. In one series of experiments, recently published, the average number of organisms per droplet of saliva as cast out in ordinary speech proved to be no less than 4375. Is not, then, the danger of infection by this means entitled to greater consideration than that more or less imaginary one of infection through sweat?

That the deeper layers of the skin yield bacteria is a well-known fact, but the mere obtaining of positive results from inoculating culture media therewith is no proof of their harmful nature. Indeed, several of the species commonly present are known to be non-pathogenic.

In speaking of these several matters, I have no intention of advocating any lessening of the precautions taken to bring the danger of infecting the wound to a minimum, but I wish to point out the inconsistency of paying so great an amount of attention to the possibility of introducing bacteria, many of them harmless, from one source and another, and neglecting that of the introduction of others, perhaps more dangerous, that are showered down through the air and projected from the mouth. I have made numerous exposures of Petri dishes to the air of operating rooms, and in every instance the great majority of the colonies produced have been of pyogenic organisms. The numbers deposited have varied widely, the maximum being 131 per square inch per hour.

In his work in the operating room, the surgeon is dependent to a great extent upon the care and thoroughness with which others, whose work he necessarily cannot supervise in every detail, carry out their part. He must assume that the dressings and sponges have been prepared with scrupulous care. He is, in a way, at the mercy of those in subordinate positions, whose carelessness or want of proper instruction may vitiate his best endeavors. To emphasize this point, I will give two illustrations:

The sterilizing plant of a hospital is to me always an attraction, and whenever I visit one I inquire into the methods followed. I was present one day in a room fitted with as perfect a lot of apparatus as one could wish to see. The person in charge had packed one of the autoclaves with dressings, which were wedged in so tightly that one could find hardly a space between them large enough to admit even a finger. On my asking what measure of sterility was expected with such solid packing, I was informed that absolute sterility would be attained in an hour, the steam being under fifteen pounds' pressure. Anybody who has ever studied steam disinfection scientifically is aware of the great necessity of loose arrangement of the objects under treatment, in order that the steam shall have as free access as possible to every part, and that the readiest means of preventing its action is solid packing.

In an autoclave in another institution, I saw a number of sponge-jars with their lids in place. I ventured to open one of them, and found that the cover fitted so tightly that some little force was necessary for its removal. I asked the attendant if the sponges therein contained were to be sterilized by steam or by dry heat, and the answer was "steam;" but it was obvious that the steam could not gain access to the interior of the jar, from which, moreover, the contained air could not escape. I received permission to test the matter practically. The contents of the jar were replaced by a number of sponges, which I infected with a twenty-four hours' old culture of *Staphylococcus pyogenes aureus.* The cover was then put on and the whole was subjected to the routine process. Cultures of the sponges were afterwards made in sterile bouillon and incubated for twenty-four hours at 37° C. The results were positive; sterilization had not been effected.

On the other hand, in some hospitals, the sterilization of dressings and sponges is carried to an unnecessary degree of thoroughness, which involves wasting much valuable time. I refer to fractional sterilization. Fractional sterilization of such materials as milk and culture media is necessary, on account of the presence of sporing organisms in a fluid rich in nutriment. The ordinary temperatures employed destroy the bacteria, but the spores resist; during the next twenty-four hours these develop into bacteria, which are destroyed by a second heating; and a third treatment insures sterility beyond a doubt. These spores, by the way, are those of certain non-pathogenic organisms; the most resistant pathogenic spores are killed by a few minutes' boiling. But in the case of dressings treated properly with steam under pressure, both the bacteria and the spores present are destroyed with one exposure, for even the very resistant non-pathogenic spores above mentioned cannot resist steam under fifteen pounds' pressure for ten minutes. But supposing they could, what effect would a second treatment on the following day have upon them? *None whatever,* for in the absence of moisture and nutrient material they could not develop into bacilli, and so long as they remained spores they would, within reasonable limits, retain their resistance. So a second exposure would be as futile as the first, and a third, fourth, and fifth would be equally barren of results.

Carried out with proper precautions as to packing and vacuum formation, sterilization of dressings and sponges requires but one exposure to steam under fifteen pounds' pressure for thirty minutes at most. Indeed, I have placed dried cultures of *Staphylococcus aureus* and *Staphylococcus albus* in the pockets and sleeves of two coats rolled loosely together, and have attained sterility in ten minutes with steam under ten pounds' pressure.

The preparation of the hands for surgical work is a subject that has interested me greatly. The potassium permanganate oxalic acid portion of the process has always excited my wonder. Three explanations have been given me of the necessity or advisability of this treatment, namely, 1. That the permanganate destroys bacteria; 2. That it oxidizes the organic matters adherent to the skin; 3. That when one stains the hands in every part with permanganate and then removes the stain with oxalic acid, the hands are clean. As to the assertion that it destroys bacteria: I have experimented with saturated permanganate solution against *Staphylococcus aureus* and *albus, Bacillus coli,* and *Bacillus pyocyaneus,* and have found that ten minutes' exposure is ineffective against all but the last mentioned. These results were obtained not once, but several times. As to the oxidation of organic matter: Potassium permanganate is pre-eminently an oxidizer of organic matter. On dipping the hands into a saturated solution, they are stained a deep brown, owing to the precipitation of a lower oxide, oxygen having been given up to the epidermal scales and other organic matter. This deposit of the lower oxide is soluble in oxalic acid, which thus restores the normal color of the hands. It is assumed that the organic matter (just what harm it will do, unless it is in the form of bacteria, I do not know) is completely oxidized and disposed of. Dip the hands, however, a second time, and see what happens. The same thing. Repeat it. Again it happens, and again, and again, and again. This is due to the fact that, immediately on contact with organic matter, the permanganate is reduced and the hands become, as it were, *plated* with the precipitated lower oxide, which acts as a bar to further action of the permanganate upon the parts immediately beneath the plating, just as the albuminate of mercury prevents the further action of corrosive sublimate. A short time ago, I tried the experiment of treating my hands first with permanganate and then with oxalic acid and repeating the process until further treatment failed to give the characteristic brown stain. On the twenty-first trial, the stain was observed to be less intense, and so on to the twenty-fifth, when the experiment was discontinued. From this it would appear that to oxidize all the organic matter possible by this means is an endless task. After twenty-five treatments, I washed my hands with soap and water, and after repeated rinsing in running water, I dipped them again into the permanganate solution. At once the original dark-brown stain appeared as intensely as ever; I was then oxidizing the traces of soap, which, in spite of continued rinsing, adheres tenaciously to the skin, as is proved by the great difficulty one observes in removing, by rinsing, the odor of a scented soap after washing therewith.

As to the statement that a hand once stained and decolorized is necessarily clean, there is but little to say. A dirty hand may be stained and decolorized as well as a clean one, but the dirt remains. Permanganate removes no dirt and destroys only weakly resistant bacteria.

After thorough brushing with hot soapsuds, what agent can be relied upon to kill the bacteria that have not been removed? Not corrosive sublimate 1–1000, if we soak the hands a quarter of an hour; not creolin 1–20, if we soak them much longer; not lysol, nor solveol, nor bacillol, nor sulphonaphtol; not peroxide of hydrogen; not sublamin; not mercuric cyanide; not even formaldehyde in 5 per cent. solution, even though the skin could stand it. All of these agents and several others I have tested under the most favorable conditions against the common pus organisms and all failed to kill within reasonable periods. Without going unnecessarily into details, I will give my results as briefly as possible. A little more than a year ago, I published the results of a series of experiments which demonstrated, among other things, that corrosive sublimate, 1–1000, requires more than ten minutes' contact to kill *Staphylococcus albus,* and that weaker solutions (1–5000) act far more slowly. Recently I tried 1–500, which solution is too strong and irritating for general application, and found that it would kill *Staphylococcus aureus* in from sixty to ninety seconds and the other pyogenic organisms in from forty to sixty seconds. With 1–100, I found that the aureus was killed after twenty seconds. Now, if 1–100 cannot destroy pus cocci in twenty seconds, and 1–500 can do so only after a minute, and 1–1000 only after ten minutes, what measure of disinfection does the surgeon attain who merely dips his hands into the solutions of corrosive sublimate in common use for only a few seconds and then rinses them off with sterile water or salt solution?

In my tests of the germicidal power of other disinfectant solutions, I employed a number of different species of bacteria; but in the brief report of results now to be made, I will deal only with that pyogenic organism which is the most common and also the most resistant,—*Staphylococcus pyogenes aureus.*

The following table shows the number of minutes that the organism mentioned remained in contact with the several disinfectants without injury, and also the shortest exposure observed that was sufficient for its destruction. The intervals adopted were as follows: Thirty seconds, 1, 2, 3, 4, 5, 7, 10, 15, 30, 45, and 60 minutes. In only one case was the exposure longer than sixty minutes, and then the agent failed to kill in three hours.

Agent.	Strength.	Failed to Kill. (Minutes.)	Killed. (Minutes.)
Carbolic acid	1–40	3	4
" "	1–20	1	2
Trikresol	1–40	2	3
"	1–20	1	2
Lysol	1–40	7	10
"	1–20	2	3
Solveol	1–40	10	15
"	1–20	10	15
Bacillol	1–40	4	5
"	1–20	3	4
Creolin	1–40	10	15
"	1–20	10	15
Sulphonaphtol	1–20	30	45
Hydrogen peroxide	Full strength	4	5

Agent.	Strength.	Failed to Kill. (Minutes.)	Killed. (Minutes.)
Formaldehyde	1 per cent.	60	. .
"	2 per cent.	30	45
"	3 per cent.	25	30
"	5 per cent.	15	20
Mercuric cyanide	1–1000	180	. .
Sublamin	1–1000	10	. .
Potassium permanganate .	Saturated	10	15
Potassium permanganate and hydrochloric acid (Audrewes)	1 per cent. each	4	5

Here, then, are twenty-two preparations, not one of which acts under two minutes, and most of them only after five.

My next endeavor was to find some preparation that will kill not in minutes, but in seconds. It was obvious that none of the organic compounds mentioned could be depended upon to do this in any strength which it would be practicable to use, and I had, therefore, to turn to the inorganic agents. Of the salts of the metals, corrosive sublimate is beyond question the most powerful, but this cannot be depended upon in its usual solutions, as I have already pointed out; and hence it must be combined with some other agent or agents, if any increase in effectiveness is to be secured. The addition of hydrochloric acid to the extent of 10 per cent. was found to be very effective, but a solution of an irritant like corrosive sublimate with another like hydrochloric acid would obviously be out of the question, on account of its local action; but if this action could be prevented, the combination might be useful. First, I experimented with a mixture which I found would kill *Staphylococcus aureus, albus,* and *citreus* and *Bacillus pyocyaneus* in less than ten seconds. Then I tried weakening it, first as to one, then as to another ingredient, but always aiming to keep its limit of required time at about ten seconds. The ninth combination tried appeared to be as weak with respect to each of the active constituents as could be made, retaining that degree of efficiency—that is, to kill the pyogenic bacteria on silk threads, not in the skin—within ten seconds. I tried it against pus from a carbuncle and against two other specimens of uncertain origin, and at the same time I tried carbolic acid and trikresol, which had proved to be the quickest in action of the twenty-two solutions above mentioned. Let me say here, that killing bacteria in pus is quite a different thing from killing them on threads and glass beads.

The carbuncle pus was killed by trikresol (1–40) in five and a half minutes and by carbolic acid (1–40) in four; my mixture killed it in less than a minute, though not in thirty seconds. The other specimens of pus were both killed by trikresol and carbolic in two minutes, and by my mixture in less than thirty seconds.

Following are the results of attempts to sterilize the skin. I have repeatedly soaked my hand (without any preliminary scrubbing) for two minutes, and then have had plantings made from material removed from about each nail and from scrapings from the skin of each finger and from the palm. Occasionally, I got a growth; but, as a rule, every tube of bouillon remained clean and sterile. A young man, whose duties included the daily cleaning of cages in the animal room and whose hands were not the subject of much thought or care, soaked his hand (after ordinary washing) on ten different occasions for from two to five minutes; and each time each nail and finger was tested, *i.e.*, ten cultures were made. In seven of the experiments there was entire absence of growths; in one, a growth was obtained from one forefinger; in one, from one middle finger; and in one, from one thumb. That is to say, of 100 plantings only three showed growths.

At the Boston City Hospital, Dr. Monks immersed his hands for two minutes without previous scrubbing; the skin of both hands gave negative results, but growths were obtained from the nails of the right hand; the nails of the left hand were sterile. Three of the assistants did the same thing, but after scrubbing, and with these results:

Dr. H. Left hand sterile; nails gave growth.
Right hand sterile; nails sterile.
Dr. K. Left hand sterile; nails sterile.
Right hand sterile; nails sterile.
Dr. S. Left hand sterile; nails sterile.
Right hand sterile; nails sterile.

Examination of the skin of Drs. H. and K. after being engaged in operating showed persistent sterility; but Dr. S.'s left hand yielded a growth. The mixture has been used also in several cases of lacerated wounds.

For my combination, I make no claims whatever, and no assertions that later might have to be recalled. I submit the facts as I have seen them. I do not say that everybody will get the same results that I have seen. To my hands and to those of my assistants, the mixture has caused no irritation beyond the same degree of biting that one notices when in contact with peroxide of hydrogen. Two of the surgical assistants reported slight exfoliation two days after trying it, but nothing more. I recognize that some skins may be more markedly irritated, and that not sufficiently numerous experiments have been made to warrant unqualified recommendation.

The composition of the mixture is as follows:

Commercial alcohol (94 per cent.)	640 cubic centimetres.
Hydrochloric acid	60 cubic centimetres.
Water	300 cubic centimetres.
Corrosive sublimate	0.8 gramme

This mixture, then, contains 60 per cent. absolute alcohol, 6 per cent. commercial (strong) hydrochloric acid, and 1–1250 corrosive sublimate. Now, 60 per cent. alcohol will destroy *Staphylococcus aureus* in four minutes; 10 per cent. hydrochloric acid is equally effective; and 1–1000 corrosive sublimate will kill it in three minutes. Why a combination containing all three substances, but with lesser proportions of the acid and the salt, is so much quicker in its action than any one of them alone, is an interesting question of physical chemistry.